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VOLUME III

ARMSTRONG LABORATORY

GENETIC TOXICITY EVALUATION OF 1,1,1,2,3,3,3-HEPTAFLUOROPROPANE VOLUME III OF III: RESULTS OF THE FORWARD MUTATION ASSAY USING L5178Y MOUSE LYMPHOMA CELLS

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January 1995

FINAL REPORT FOR THE PERIOD MARCH THROUGH DECEMBER 1994

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TECHNICAL REVIEW AND APPROVAL

AL/OE-TR-1995-0008
VOLUME III

The experiments reported herein were conducted according to the "Guide for the Care and Use of Laboratory Animals," Institute of Laboratory Animal Resources, National Research Council.

This report has been reviewed by the Office of Public Affairs (PA) and is releasable to the National Technical Information Service (NTIS). At NTIS, it will be available to the general public, including foreign nations.

This technical report has been reviewed and is approved for publication.

FOR THE COMMANDER

TERRY A. CHILDRESS, Lt Col, USAF, BSC
Director, Toxicology Division
Armstrong Laboratory

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13. ABSTRACT (Maximum 200 words) Under subcontract to ManTech Environmental Technology, Incorporated, Genesys Research, Incorporated used L5178Y mouse lymphoma cells from clone 3.7.2C to assess the capability of 1,1,1,2,3,3,3-heptafluoropropane (HFC-227ea) to induce gene and chromosomal mutations at the thymidine kinase (*tk*) locus in the absence and presence of exogenous S9 metabolic activation.

The provided MSDS for HFC-227ea indicated that no information was available on the solubility of HFC-227ea in water. To test this volatile material, cell cultures were placed in 15 ml round-bottom glass blood tubes sealed with serum stoppers, and, using a syringe, predetermined volumes of air were removed from each tube and nominal concentrations of HFC-227ea material were introduced. Three tubes were used for each concentration, a tube containing 5 ml of cells in media without metabolic activation, a tube containing 5 ml of cells in media plus the metabolic activation mixture, and a "sham" tube containing only 5 ml of medium. After a four-hour exposure period at 37°C, the sham tubes were allowed to cool to room temperature, and the concentrations of the test material were measured using infrared (IR) analysis.

HFC-227ea was tested in a concentration range-finding assay and a mutagenesis assay, with each assay conducted in the absence and presence of metabolic activation. No concentration-related increases in toxicity were obtained in either assay of HFC-227ea. These results suggest that HFC-227ea may have been insoluble in cell culture medium. In the mutagenesis assay, the negative control cloning efficiencies and spontaneous mutation frequencies met the criteria for acceptability, and positive control mutant frequencies were within the historical ranges for the laboratory. When tested to the maximum concentration that could be obtained under the conditions of testing, HFC-227ea was negative with no toxicity (=) in the L5178Y/*tk* $^{+/-}$ mouse lymphoma cell mutagenesis assay. Therefore, HFC-227ea did not induce gene or chromosomal mutations in mammalian cells *in vitro*.

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PREFACE

The U.S. Air Force is investigating chemical replacements for the fire suppressant/extinguishant Halon 1301. 1,1,1,2,3,3,3-heptafluoropropane (HFC-227ea) has excellent solvent properties and may serve as a "drop in" extinguishant replacement. Results from laboratory animal *in vivo* studies indicate that HFC-227ea has a low order of acute toxicity. A comprehensive literature search indicated that no information was available on the mutagenic potential of.HFC-227ea. ManTech Environmental initiated a battery of three short-term assays that were utilized to assess the mutagenic and clastogenic potential of HFC-227ea. Protocols for these assays were in conformance with the Environmental Protection Agency's (Toxic Substances Control Act) Health Effects Testing Guidelines.

This document, Volume III of III, serves as a final report detailing the results of the forward mutation assay using L5178Y mouse lymphoma cells. Volumes I and II describe, respectively, the results of the *salmonella typhimurium* histidine reversion assay (Ames assay) and the *in vivo* mouse bone marrow erythrocytes micronucleus testing.

The research described herein began in March 1994 and was completed in December 1994 by Genesys Research, Inc., Research Triangle Park, NC under a subcontract to ManTech Environmental Technology, Inc., Toxic Hazards Research Unit (THRU), and was coordinated by Darol E. Dodd, Ph.D., THRU Laboratory Director. This work was sponsored by the Toxicology Division, Occupational and Environmental Health Directorate, Armstrong Laboratory, and was performed under Department of the Air Force Contract No. F33615-90-C-0532 (Study No. F30). Lt Col Terry A. Childress served as Contract Technical Monitor for the U.S. Air Force, Armstrong Laboratory, Toxicology Division.

SUMMARY

Under subcontract to ManTech Environmental Technology, Incorporated, Genesys Research, Incorporated used L5178Y mouse lymphoma cells from clone 3.7.2C to assess the capability of 1,1,1,2,3,3,3-heptafluoropropane (HFC-227ea) to induce gene and chromosomal mutations at the thymidine kinase (*tk*) locus in the absence and presence of exogenous S9 metabolic activation.

The provided MSDS for HFC-227ea indicated that no information was available on the solubility of HFC-227ea in water. To test this volatile material, cell cultures were placed in 15 ml round-bottom glass blood tubes sealed with serum stoppers, and, using a syringe, predetermined volumes of air were removed from each tube and nominal concentrations of HFC-227ea material were introduced. Three tubes were used for each concentration, a tube containing 5 ml of cells in media without metabolic activation, a tube containing 5 ml of cells in media plus the metabolic activation mixture, and a "sham" tube containing only 5 ml of medium. After a four-hour exposure period at 37°C, the sham tubes were allowed to cool to room temperature, and the concentrations of the test material were measured using infrared (IR) analysis.

HFC-227ea was tested in a concentration range-finding assay and a mutagenesis assay, with each assay conducted in the absence and presence of metabolic activation. No concentration-related increases in toxicity were obtained in either assay of HFC-227ea. These results suggests that HFC-227ea may have been insoluble in cell culture medium. In the mutagenesis assay, the negative control cloning efficiencies and spontaneous mutation frequencies met the criteria for acceptability, and positive control mutant frequencies were within the historical ranges for the laboratory. When tested to the maximum concentration that could be obtained under the conditions of testing, HFC-227ea was negative with no toxicity (=) in the L5178Y/$tk^{+/-}$ mouse lymphoma cell mutagenesis assay. Therefore, HFC-227ea did not induce gene or chromosomal mutations in mammalian cells *in vitro*.

GENESYS RESEARCH INCORPORATED'S GOOD LABORATORY PRACTICE COMPLIANCE STATEMENT

IN VITRO FORWARD MUTATION ASSAY OF 1,1,1,2,3,3,3-HEPTAFLUOROPROPANE (HFC-227ea) USING THE L5178Y/*tk*$^{+/-}$ MOUSE LYMPHOMA CELL MUTAGENESIS ASSAY (MLA) WITH COLONY SIZING, WITH AND WITHOUT METABOLIC ACTIVATION

Genesys Research Incorporated's portion of the above titled study was reviewed for compliance with Quality Assurance (QA) regulations and with the provisions of the United States Environmental Protection Agency/Toxic Substances Control Act Good Laboratory Practice (GLP) Standards as defined in the Federal Register, August 17, 1989 (40 CFR, Part 792) and TSCA Test Guidelines, Federal Register, September 27, 1985 (Vol. 50, #188, Part 798.5265) and its revision (May 20, 1987, Vol. 52, #97).

The practices used in the study were found to be in compliance with these regulations.

Ann D. Mitchell 12/17/94

Ann D. Mitchell, Ph.D. Date
Study Director

GENESYS RESEARCH INCORPORATED'S QUALITY ASSURANCE STATEMENT

With the exception of the handling, storage, dilution (for exposure of the cells) and analytical chemistry of the test material, which were the responsibility of ManTech Environmental Technology, Incorporated, the data and the report for the following study carried out at Genesys Research, Incorporated has been reviewed and approved for compliance with the provisions of the United States Environmental Protection Agency/Toxic Substances Control Act Good Laboratory Practice (GLP) Standards as defined in the Federal Register, August 17, 1989 (40 CFR, Part 792) and TSCA Test Guidelines, Federal Register, September 27, 1985 (Vol. 50, #188, Part 798.5265) and its revision (May 20, 1987, Vol. 52, #97).

The final report accurately describes the methods that were used and accurately reflects the raw data of the study.

ManTech Environmental Technology Incorporated Study Number: 1093-F30

Genesys Research, Incorporated Study Number: 94036

Type Study: L5178Y/$tk^{+/-}$ Mouse Lymphoma *In Vitro* Mammalian Cell Mutagenesis Assay

Protocol Signed by Study Director: March 19, 1994

Date Testing Started: May 13, 1994

Critical Phase Audit(s): April 20 and May 17, 1994

Date Testing Completed: June 6, 1994

Date Draft Report Audited: September 25 and October 1, 1994

Date Audit Findings Reported to Management: April 20, May 17 and October 1, 1994

Approved: Helen M. King

Helen M. King, B.S.
Quality Assurance Officer for Genesys

Date: 12/17/94

MANTECH ENVIRONMENTAL TECHNOLOGY, INCORPORATED GOOD LABORATORY PRACTICE COMPLIANCE STATEMENT

Study Title: *In Vitro* and Inhalation Toxicity Study of 1,1,1,2,3,3,3-Heptafluoropropane

Project Number: 1093-F30

Study Director: Allen Ledbetter

ManTech Environmental Technology's portion of this study was conducted in accordance with EPA Good Laboratory Practice Regulations (GLP) as set forth in the Code of Federal Regulations (40 CFR 792). There were no significant deviations, in the work conducted by ManTech, from the aforementioned GLP regulations that would have affected the integrity of the study or the interpretation of the test results. The ManTech generated raw data have been reviewed by the Study Director, who certifies that the information contained in this report represents an appropriate and accurate conclusion within the context of the study design and evaluation criteria. Deviations are listed below:

1. The sponsor was responsible for the test substance characterization, stability and homogeneity analysis.

All original ManTech generated raw data are retained in the ManTech Environmental Technology's Archives, at 5 Triangle Drive, Research Triangle Park, NC 27709, with a copy of the final study report.

SUBMITTED BY:

Study Director:

Allen Ledbetter · 12/16/94

Allen Ledbetter Date

MANTECH ENVIRONMENTAL TECHNOLOGY, INCORPORATED QUALITY ASSURANCE STATEMENT

Study Title: *In Vitro* and Inhalation Toxicity Study of 1,1,1,2,3,3,3-Heptafluoropropane

Project Number: 1093-F30

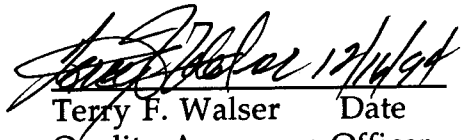
Study Director: Allen Ledbetter

Report Audit Dates:

This study has been subjected to inspections and the report has been audited by ManTech Environmental Technology's Quality Assurance Unit. The report describes the methods and procedures used in the study and the reported results accurately reflect ManTech's raw data. ManTech's raw data and a copy of the final report will be stored in room 210 in the MET building at Research Triangle Park, NC. The sponsor was responsible for the Iodotrifluoromethane characterization, stability and homogeneity analyses.

The following are the inspection dates, and the dates inspection reports were submitted:

Phase(s)	Date(s) of Inspection	Report Submitted to Study Director	Report Submitted to Management
Protocol	8/2/94	8/2/94	8/2/94
(Partial) Data Review	11/23/94		
Data Review	12/15/94	12/16/94	12/16/94



Terry F. Walser Date
Quality Assurance Officer

IN VITRO FORWARD MUTATION ASSAY OF 1,1,1,2,3,3,3-HEPTAFLUOROPROPANE (HFC-227ea) USING THE L5178Y/$tk^{+/-}$ MOUSE LYMPHOMA CELL MUTAGENESIS ASSAY (MLA) WITH COLONY SIZING, WITH AND WITHOUT METABOLIC ACTIVATION

1. INTRODUCTION

Under subcontract to ManTech Environmental Technology, Incorporated (ManTech), Dayton, Ohio (ManTech/Dayton) Genesys Research, Incorporated (Genesys) used L5178Y mouse lymphoma cells from clone 3.7.2C to assess the capability of 1,1,1,2,3,3,3-heptafluoropropane (HFC-227ea) to induce gene and chromosomal mutations at the thymidine kinase (*tk*) locus in the absence and presence of exogenous S9 metabolic activation. Allen Ledbetter, ManTech Environmental Technology, Incorporated, Research Triangle Park, North Carolina (ManTech/RTP), was responsible for handling, storage, dilution (for exposure of the cell cultures), and analytical chemistry of the test material.

Testing at Genesys consisted of all procedures not performed by ManTech/RTP and was conducted under the direction of Ann D. Mitchell, Ph.D., Study Director, by J. Thom Deahl, M.S., and Diane M. Brecha, B.S., Genetic Toxicologists, in accordance with the provisions of the United States Environmental Protection Agency/Toxic Substances Control Act Good Laboratory Practice (GLP) Standards as defined in the Federal Register, August 17, 1989 (40 CFR, Part 792) and TSCA Test Guidelines, Federal Register, September 27, 1985 (Vol. 50, #188, Part 798.5265) and its revision (May 20, 1987, Vol. 52, #97). Testing was initiated with a preliminary concentration range-finding assay on May 13, 1994 and concluded on June 6, 1994 with colony sizing for the mutagenesis assay of HFC-227ea. The protocol, a protocol amendment, raw data obtained by Genesys, and a copy of this report will be retained in Genesys' archives located at 2300 Englert Drive, Durham, NC 27713.

2. BACKGROUND

The development of the L5178Y mouse lymphoma cell mutagenesis assay (MLA) in the early 1970's by Clive and associates and further changes in the assay during the past two decades have been described by Clive *et al.* (in preparation).

The MLA detects mutations affecting the heterozygous thymidine kinase (*tk*) locus of L5178Y/$tk^{+/-}$-3.7.2C mouse lymphoma cells. In the MLA, two classes of mutants, both detected by their ability to form colonies in the presence of trifluorothymidine (TFT), which is toxic to $tk^{+/-}$ cells, can be distinguished based on colony size: large (λ) colony mutants which have normal 11b chromosomes, the site of the *tk* locus, and small (σ, slowly growing) colony mutants which often have cytogenetic damage to chromosome 11b that can be detected in conventionally-stained and banded chromosomes (Blazak *et al.*, 1986; Clive *et al.*, 1980; Hozier *et al.*, 1981, 1982; Moore *et al.*, 1985). Hence, chemicals that induce large numbers of σ colony mutants are generally considered to be clastogens (chromosome-breaking chemicals, Moore *et al.*, 1985). This feature distinguishes the

L5178Y mouse lymphoma assay from mammalian cell mutagenesis assays that measure effects at hemizygous loci (such as *hprt*, *aprt*+/o, or *tk*+/o), as the latter cannot detect slowly growing mutants (DeMarini *et al.* , 1989).

Because chemicals can induce a continuous spectrum of genetic damage, from alterations at the molecular level to extensive damage (including deletions, chromosomal breakage and rearrangements) before toxic levels are reached, it is necessary to maximize σ colony mutant recovery in the MLA in order to obtain complete information on this spectrum of genetic events.

3. METHODS

3.1. Identification, Storage, and Dilution of the Test Materials

The test material, 1,1,1,2,3,3,3-heptafluoropropane (HFC-227ea, or F_7C_3H; molecular weight 170; CAS Number 431-89-0), a colorless gas, was received in a steel gas container from ManTech/Dayton on April 7, 1994 then transferred to Allen Ledbetter, ManTech/RTP, who was responsible for handling, storage, and dilution of the test material. The HFC-227ea was stored at ManTech/RTP at room temperature (approximately 72°F). ManTech/Dayton documented the strength, purity, and composition of the test material and provided a Material Safety and Data Sheet (MSDS) from Great Lakes Chemical Corporation for HFC-227ea. Upon acceptance of the final report, the remaining test material will be returned to the Sponsor. No reserve sample will be retained by ManTech/RTP.

3.2. Controls

The positive control chemicals were hycanthone (HYC, CAS No. 3105-97-3), a chemical that induces mutagenesis, with predominantly σ mutants, in the absence of an exogenous metabolic activation system, and cyclophosphamide (CP, CAS No. 50-18-0), a chemical that induces mutagenesis, with predominantly σ mutants, only with exogenous metabolic activation. The negative control was air. For assays using metabolic activation, the activation mixture was also added to the controls.

3.3. Metabolic Activation Preparations

Rat liver S9 homogenate, in KCl buffer, prepared from Aroclor 1254-induced male Sprague-Dawley rats, was obtained from Molecular Toxicology, Inc., Annapolis, Maryland and stored frozen in liquid nitrogen. It was thawed and used to prepare an S9 mixture immediately before the chemical exposure step of each assay. The final concentrations of the S9 mixture for the preliminary concentration range-finding assay included 2.4 mg/ml NADP and 4.5 mg/ml sodium isocitrate, which were prepared in serum-free medium, pH adjusted to 7.0 with 1N NaOH, and filter sterilized before adding 50 µl/ml S9 homogenate, then adding the mixture to the cells in F_{5HP} medium. To reduce potential toxicity of the S9 mixture for the mutagenesis assay, the NADP con-

centration was reduced to 1.2 mg/ml and the S9 homogenate was reduced to 25 µl/ml. All other components were unchanged.

3.4. Cell Culture

L5178Y mouse lymphoma cells, clone 3.7.2C, provided by Dr. Donald Clive, Burroughs Wellcome Co., Research Triangle Park, NC, are stored in liquid nitrogen at Genesys. The cells were grown as a suspension culture in F_{10HP} medium (see composition below), cleansed of homozygous ($tk^{-/-}$) cells with medium containing 0.1 µg/ml methotrexate, as described by Mitchell *et al.*, 1988, and used as target cells for chemical exposure.

3.5. Media

L5178Y cells were cultivated in Fischer's medium for leukemic cells of mice supplemented with 31 µg/ml penicillin (1650 units/mg), 50 µg/ml streptomycin sulfate, 0.1% Pluronic F68, 0.22 mg/ml sodium pyruvate, 25 mM HEPES buffer, and 10% heat-inactivated horse serum to make F_{10HP}. F_{5HP}, containing 5%, rather than 10%, heat-inactivated horse serum, was the medium used for exposures in the presence of exogenous metabolic activation. F_{10P} was the medium used for exposures in the absence of exogenous metabolic activation and F_{10P} was used during the expression period. The horse serum concentration was 20% in incomplete cloning medium (ICM), which did not contain agar. BBL agar (0.22%, final concentration) was added to ICM to make complete cloning medium (CCM). The selective cloning medium contained TFT at a final concentration of 1 µg/ml.

3.6. Exposure of Cell Cultures

For testing this volatile material in the preliminary concentration range-finding and mutagenesis assays, three sterile 15 ml round-bottom glass blood tubes, sealed with red rubber serum stoppers, were prepared for each concentration level: a tube for the culture tested without activation, a tube for the culture tested with activation, and a sham tube that contained medium only (no cells or S9) which was used to estimate post-exposure infrared (IR) analysis of the concentration of test material in the other two tubes.

To provide a maximum available volume for the test material, each culture contained approximately 2.5×10^6 cells in 5 ml of F_{10HP} for cultures tested without exogenous metabolic activation, or in 1.5 ml S9 mix plus 3.5 ml F_{5HP} for cultures tested with metabolic activation. Therefore, at least 10 ml/tube was available for the volatile test material. After the cultures had been placed in the tubes at Genesys and the stoppers replaced, the tubes to be exposed to HFC-227ea were transported to ManTech/RTP where, using a syringe, a predetermined volume of air was withdrawn from each tube and an equal volume of the pure test material was added. (This was not necessary for the negative control [air] or the positive controls; the latter were added directly to the cell cultures before the tubes were sealed.) The three tubes per test material concentra-

tion were then returned to Genesys, and exposure was initiated by placing them in a roller drum, and rotating them (~40 rpm) for 4 hours at 37°C.

After the exposure period, the cultures containing cells tested without and with S9 were transferred from the blood tubes to 15 ml plastic centrifuge tubes, for subsequent steps in the assays. The sham tubes were allowed to cool to room temperature prior to analysis of the concentrations of the test materials by IR.

3.7. IR Calibration and Analysis

The IR instrument (Miran 1A, Foxboro Corp., Foxboro, MA; operated with wavelength = 9.7 microns, pathlength = 6.75 meters, absorbance = 0.25, slit = 1, and range = X1) was calibrated using a "closed-loop" method prior to analyzing the sham tube atmospheres. Calibration curves were prepared (using a TI-60 calculator, Texas Instruments, Lubbock, Texas) for concentration versus recorder chart lines using the least-squares method. Due to the wide concentration ranges of HFC-227ea in the tubes (10% - 90%, 1% = 10,000 ppm) in each assay, a single calibration curve was prepared for each assay, and the volume analyzed from each tube was varied based upon the expected tube concentration. The atmospheres were analyzed by withdrawing a volume of the atmosphere from the tube with a gas-tight syringe and injecting the sample into the IR instrument, which was in the "close-loop" configuration. The number of chart lines was entered into the calculator, and the corresponding concentration obtained. The concentration was then corrected for the injection volume.

3.8. Preliminary Range-Finding Assay

One range-finding assay of HFC-227ea was conducted, with and without metabolic activation, to determine the most effective concentrations of HFC-227ea to use in the mutagenesis assay. A series of 9 nominal (calculated theoretical) concentrations of HFC-227ea were used in the preliminary assay. The procedures followed were the same as for the mutagenesis assays (described below) except that the cells were not cloned.

For each culture, growth of the cells in suspension (SG) was calculated each day by dividing the cell concentration at the end of that time period by the initial cell concentration. Total suspension growth (TSG) was calculated by multiplying day one SG by day two SG; relative suspension growth (RSG) was calculated by dividing TSG of each culture by the average TSG of the medium controls. The results from these experiments were then evaluated to select concentrations for mutagenesis testing.

3.9. Mutagenesis Assay

After exposure of the cultures as described above, the cells were centrifuged at low speed (~250 x *g*) for 5 minutes and the supernatant removed. The cells were rinsed at least twice by resuspension and centrifugation in F_{10HP} medium and then resuspended in 10 ml of F_{10HP} for growth during a two-day expression period.

During the expression period, the cell density was determined each day, and cells were diluted as necessary to maintain an optimum growth rate. On the second day of each assay, cultures were selected for cloning and 3×10^6 cells were removed from each of the test material, negative and positive control cultures to be cloned. An aliquot of 1000 cells was then obtained from each of these cultures by serial dilution and was cloned to determine cloning efficiency. After adding 1 μg/ml of TFT, the remainder of the 3×10^6 cells from each culture were cloned to determine mutant frequency. The soft-agar cloning medium was allowed to gel at room temperature for 15-20 minutes, then the dishes containing the cells were placed in a humidified CO_2 incubator and incubated at 37°C for 14 days.

Colonies in the mutant count and cloning efficiency dishes were counted, and the colonies in the mutant count plates were sized, as described below, using an Artek 982B semi-automatic colony counter with a high resolution video camera. For each culture, the absolute cloning efficiency (CE) was calculated by dividing the number of colonies in the cloning efficiency dishes by the number of cells cloned to measure cloning efficiency; relative cloning efficiency (RCE) was calculated by dividing the CE of each culture by the average CE of the negative (medium) controls, and relative total growth (RTG) was obtained by multiplying RSG by RCE. The mutation frequency (MF) for each culture was calculated by dividing the number of mutant colonies by the number of cells plated and multiplying by the reciprocal of the cloning efficiency. The average mutation frequency of the solvent control cultures was subtracted from that of each treated sample to express each result as an induced mutation frequency (IMF).

3.10. Colony Size Analysis

Mutant colony size distribution measurements were made using the semi-automatic stepping function of the Artek 982B counter. For test materials yielding a positive response, the small, large, and total mutation frequencies are reported for each treated sample. For test materials yielding a negative response, the small, large, and total mutation frequencies are reported for the positive and negative controls.

3.11. Raw Data Collection

All observations, raw data collected, and calculations were recorded onto standard forms which were bound together with the study protocol at the conclusion of testing.

3.12. Analysis and Interpretation of Results

a. Data Collected

Experimental data collected for each sample included growth in suspension, cloning efficiency, and mutant counts. From these data were calculated relative total growth (defined in Mitchell *et al.*, 1988), absolute cloning efficiency of the medium controls, and total mutation frequencies. Small and large colony mutation frequencies were obtained for each sample that was sized as described above. For acceptable experiments,

the cloning efficiencies of the solvent controls should be at least 70%, the average spontaneous (negative control) mutation frequencies should be $\geq 50 \times 10^{-6}$, and the positive control mutation frequencies should be within the historical ranges for the laboratory.

b. Criteria for Interpretation

The results were evaluated according to the categories of responses utilized by the U.S. EPA Gene-Tox Workgroup (Mitchell *et al.*, in preparation), as follows:

++ Strong positive response with evidence of a dose-response and an induced mutation frequency of at least 100×10^{-6} (Δ_{100}) at a relative total growth (RTG) $\geq$ 20%.

\+ Positive response with evidence of a dose-response and an induced mutation frequency of at least 70×10^{-6} (Δ_{70}) at a RTG $\geq$ 10%.

\- Negative response for which toxicity is evidenced by a RTG of 10 - 20%, and the positive control mutation frequency demonstrates that there are no inherent problems with the assay.

= Negative response with no toxicity, and the positive control mutation frequency demonstrates that there are no inherent problems with the assay.

E Equivocal response in which positive and negative results are obtained in repeated experiments, and no reason is found to give greater weight to the positive or the negative result.

\# Not-testable. The test material can not be tested to sufficiently high concentration to obtain a conclusive result in the MLA because of limited solubility, acidic pH shifts, the test material's dissolving plastic, etc.

Hence, biological significance was considered in the evaluation of the results. The final interpretation of the results was the responsibility of the Study Director.

4. RESULTS AND DISCUSSION

Summaries of the concentrations tested, results obtained in preliminary concentration range-finding experiments, and the results obtained for testing HFC-227ea in the L5178Y/*tk*$^{+/-}$ mammalian cell mutagenesis assays are presented in Tables 1 - 3.

4.1. Concentrations Tested

Table 1 summarizes the initial nominal concentrations of HFC-227ea in the sets of tubes without and with activation for each assay, the IR determined concentrations from the corresponding sham tubes, and the percent of test material recovered after the exposure periods. As illustrated in Table 1, ~57 to 74% of the test material was recovered

in the preliminary assay, and ~63 to 91% recovery was obtained in the mutagenesis assay.

Differences in the nominal and IR determined concentrations could have arisen from: (a) lack of precision in providing the nominal concentrations; (b) leakage of the test materials from the tubes during the exposure period; or (c) absorption of the test materials by the cell culture medium. Lack of precision and leakage are considered to have been minimal because of the relatively small variability in percent recovery for most samples in each assay. The provided MSDS stated that information on the solubility of HFC-227ea in water was not available.

4.2. HFC-227ea Results

As summarized in Table 1, HFC-227ea was tested over a nominal concentration range of 100,000 to 900,000 ppm, which resulted in IR determined concentrations of up to 538,535 and 568,484 ppm, respectively, in the preliminary concentration range-finding and the mutagenesis assay. But, as illustrated in Tables 2 and 3, no depressions in RSG were obtained for any culture exposed to HFC-227ea in either assay.

Because in the preliminary assay (Table 2) HFC-227ea was tested over a range of nine concentrations, up to the maximum concentration that could be obtained under the conditions of testing, with no evidence of toxicity, the mutagenesis assay was conducted over a similar range of concentrations, but with only five, more widely spaced, concentrations.

In the mutagenesis assay of HFC-227ea (Table 3), the average absolute cloning efficiencies of the negative controls (air) were 104.7% in the absence of activation and 127.5% in the presence of metabolic activation (data not shown); the spontaneous mutation frequencies were 62 x 10^{-6} in both the absence and presence of activation. Therefore, the negative control cloning efficiencies and the spontaneous mutation frequencies met the criteria for acceptability. Positive control mutant frequencies were within the historical ranges for the laboratory. In the absence of activation, 10.0 µg hycanthone/ml yielded an IMF of 681 x 10^{-6} at 7.1% RTG, and, in the presence of activation, 2.5 µg cyclophosphamide/ml yielded an IMF of 310 x 10^{-6} at 21.0% RTG. For both positive controls, primarily small (σ) colony mutants were produced.

As illustrated in Table 3, no concentration of HFC-227ea was mutagenic. Therefore, the results obtained in testing HFC-227ea in the L5178Y/*tk*$^{+/-}$-mouse lymphoma cell mutagenesis assay are evaluated as = which is defined as a negative response with no toxicity in which the positive control mutation frequency demonstrates that there were no inherent problems with the assay. Thus, when tested to the maximum concentrations that could be obtained under the conditions of testing, 1,1,1,2,3,3,3-heptafluoropropane (HFC-227ea) did not induce gene or chromosomal mutations in mammalian cells *in vitro*.

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Table 1: Comparison of Nominal and IR Determined Percent Recovered[a] Concentrations of 1,1,1,2,3,3,3-Heptafluoropropane (HFC-227ea) in the Preliminary Concentration Range-Finding Assay and the L5178Y/*tk*$^{+/-}$ Mouse Lymphoma Mammalian Cell Mutagenesis Assay.

Nominal Concentration[b] (ppm)	IR Determined Concentration[c] (ppm)	Percent Recovered[d]
Preliminary Assay		
100,000	63,967	64.0
200,000	147,112	73.6
300,000	204,380	68.1
400,000	269,853	67.5
500,000	344,792	69.0
600,000	378,811	63.1
700,000	423,733	60.5
800,000	458,673	57.3
900,000	538,535	59.8
Mutagenesis Assay		
100,000	84,765	84.8
300,000	274,259	91.4
500,000	408,759	81.8
700,000	518,570	74.1
900,000	568,484	63.2

a The same concentrations were used for cultures tested in the absence and presence of metabolic activation.
b Initial concentrations in tubes containing cells in culture medium (without and with metabolic activation) and the sham tubes containing medium.
c Concentration in the sham tube after the exposure period.
d (Concentration in sham tube after exposure + initial concentration in sham tube and tubes containing cell cultures) x 100.

Table 2: Relative Suspension Growth of L5178Y Cell Cultures Exposed to Increasing Concentrations of 1,1,1,2,3,3,3-Heptafluoropropane (HFC-227ea) in the Absence and Presence of Activation in the Concentration Range-Finding Assay.

Chemical	+/- S9	Concentration*	RSG (%)
Air	-	N/A	104.51
Air	-	N/A	95.49
HFC-227ea	-	100,000 ppm	94.17
HFC-227ea	-	200,000 ppm	114.49
HFC-227ea	-	300,000 ppm	97.31
HFC-227ea	-	400,000 ppm	98.29
HFC-227ea	-	500,000 ppm	112.06
HFC-227ea	-	600,000 ppm	98.88
HFC-227ea	-	700,000 ppm	92.61
HFC-227ea	-	800,000 ppm	105.35
HFC-227ea	-	900,000 ppm	135.04
Air	+	N/A	89.78
Air	+	N/A	110.22
HFC-227ea	+	100,000 ppm	108.14
HFC-227ea	+	200,000 ppm	114.35
HFC-227ea	+	300,000 ppm	116.51
HFC-227ea	+	400,000 ppm	93.05
HFC-227ea	+	500,000 ppm	92.98
HFC-227ea	+	600,000 ppm	89.77
HFC-227ea	+	700,000 ppm	87.45
HFC-227ea	+	800,000 ppm	98.88
HFC-227ea	+	900,000 ppm	111.28

* = Nominal Concentration; RSG(%) = Percent relative suspension growth.

Table 3: Results from the L5178Y/$tk^{+/-}$ Mouse Lymphoma Mammalian Cell Mutagenesis Assay of 1,1,1,2,3,3,3-Heptafluoropropane (HFC-227ea) in the Absence and Presence of Metabolic Activation.

Chemical	+/- S9	Conc.*	RSG(%)	RTG (%)	MF x 10^{-6}	IMF x 10^{-6}	Notes
Air	-	N/A	115.40	103.47	63		
Air	-	N/A	84.60	93.35	61		
HFC-227ea	-	100,000 ppm	80.65	-**	-**	-**	
HFC-227ea	-	300,000 ppm	103.36	-**	-**	-**	
HFC-227ea	-	500,000 ppm	93.64	90.41	53	-	
HFC-227ea	-	700,000 ppm	89.60	87.86	47	-	
HFC-227ea	-	900,000 ppm	105.31	83.43	66	4	
Hycanthone	-	7.50 µg/ml	35.06	7.81	648	586	◆◆
Hycanthone	-	10.00 µg/ml	23.34	7.12	743	681	◆◆
Air	+	N/A	115.72	103.89	63		
Air	+	N/A	84.28	92.90	61		
HFC-227ea	+	100,000 ppm	94.81	-**	-**	-**	
HFC-227ea	+	300,000 ppm	121.33	-**	-**	-**	
HFC-227ea	+	500,000 ppm	104.61	89.58	65	2	
HFC-227ea	+	700,000 ppm	119.68	100.50	47	-	
HFC-227ea	+	900,000 ppm	125.86	99.09	37	-	
Cyclophosphamide	+	2.50 µg/ml	65.86	21.00	372	310	◆◆
Cyclophosphamide	+	3.75 µg/ml	29.85	5.33	518	455	◆◆

* = Nominal Concentration; RSG(%) = Percent relative suspension growth; RTG(%) = Percent relative total growth; MF = mutant frequency; IMF = induced mutant frequency; ◆◆ = IMF $\geq 100 \times 10^{-6}$; **Not cloned for mutagenesis.

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